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13. ABSTRACT (Maximum 200 Words)

Breast cancer risk for both sporadic and familial breast cancer can better be elucidated in studies in women with *BRCA1* mutations. Since the BRCA1 protein is involved in DNA repair, genotype-phenotype relationships can be established by the assessment of DNA repair efficiency and the identification of genetic polymorphisms in the BRCA1 DNA repair pathway. To study risks associated with certain polymorphisms in DNA repair genes, EBV-immortalized cells from women in the LCC Registry were used in both a phenotypic assay that measures DNA repair capacity and genotypic assays that determined genotypes that increased susceptibility to breast cancer. Ninety-five affected and 65 healthy BRCA1 mutation carriers are being analyzed in this study. Gamma radiation was used to induce chromosomal breaks and those chromosomal breaks were scored, counted and compared within each patient and between patients. Also, the *BRCA1*, *BRCA2*, and *Rad51* genes are being sequenced and the genotypes whose polymorphisms code for amino acid changes in conserved and functional areas are being analyzed. We predict that polymorphisms in these genes will be highly correlated with deficient DNA repair in high risk breast cancer families. Several *BRCA1* and *BRCA2* polymorphisms appear to be associated with decreased DNA repair efficiency. Specifically, the N372H (OR: 7.0 CI: 0.3-140.0) polymorphism in BRCA2, the K1183R (OR:1.8 CI:0.1-17.7) polymorphism in BRCA1, and the 5382insC mutation (OR:3.0 CI:0.3-29.8), appear to be correlated with mutagen sensitivity, but a larger sample size is needed for odds ratios to be significant. In addition, we have a large population of 187delAG (n=54) and 5382insC (n=21) mutation carriers, so we can begin to look at what polymorphisms will affect risk within those mutation carriers. Ultimately, the study of the high risk families can inform, and set priorities for the study of genetic polymorphisms in sporadic breast cancer studies.

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Introduction

Breast cancer is the second leading cause of cancer death and is the most common cancer in women living in the US (1). Breast cancer risk for both sporadic and familial breast cancer can be better elucidated in some women through the study of cancer risk in women from high-risk families. In 1994, *BRCA1* was isolated (2) and it is now recognized that women with *BRCA1* mutations have an increased risk of invasive breast and ovarian cancer. Specifically, inherited mutations in *BRCA1* are associated with a 56-87% risk of breast cancer before the age of 70 (3,4), the lower estimate or risk representing an unselected population of individuals and the higher values are those with a strong family history of cancer. However, the determinants of why some women with mutations develop breast cancer (i.e. the penetrance) and others do not is mostly unknown. We hypothesize that genetic polymorphisms play an important role in both sporadic and hereditary breast cancer risk. The factors that regulate who actually gets breast cancer in women with highly penetrant mutations are only now being identified and it is possible that the same genetic traits and exposures trigger breast cancer in the general population; *BRCA1* mutation carriers are just substantially more susceptible to these gene-environment interactions. The study of penetrance in *BRCA1* mutation carriers, therefore, might identify potential sporadic breast cancer risk factors and provide for prioritization for study in the general population. Although, it is generally believed that this is due to dietary, lifestyle and environmental exposures, heritable traits and genetic susceptibilities may play a significant role. There are countless genetic polymorphisms that may modulate hereditary and sporadic breast cancer risk, but little guidance on which ones to study. Many polymorphisms have been identified, but the relationship between the allelic variant and its possible change in protein function is usually unknown. This study will utilize a unique resource and capitalize on a high risk group of women to provide important information about high-risk and sporadic breast cancer. Specifically, this study will be able to answer these questions:

1. Why do only some women that harbor the BRCA1 mutation get breast cancer and others don't?
2. Are there polymorphisms in (involved in DNA repair) genes that increase susceptibility or modify breast cancer risk in this group?
3. How would this apply to sporadic breast cancer patients? Will that polymorphism increase risk of breast cancer in the general population?

The significance of this study lies in ascertaining how polymorphisms in DNA repair genes affect *BRCA1* penetrance and how this information could be applied to sporadic breast cancer. This will allow for prioritizing which genes to assess in epidemiological studies, and also provide an indication about which ones might have the greatest effect and interaction with *BRCA1*. The identified polymorphisms will then be tested in an existing epidemiological breast cancer study, a funded NIH and DOD case-control study of breast cancer from the University of Buffalo that studies the relationship between alcohol, diet, oxidative damage and sporadic breast cancer. Ultimately, these studies will identify susceptible groups of women and aid in the development of more rationale prevention strategies.

DNA repair and the DNA repair complex

Genes that affect *BRCA1* mutation penetrance might be those involved in DNA repair, because BRCA1 is itself believed to be involved in DNA repair. At least four pathways of DNA repair operate on specific types of damaged DNA, and each pathway involves numerous molecules. *BRCA1* is involved in double-stranded break repair; there are two different pathways involved in double-strand break repair: homology-directed repair or homologous recombinational repair (HR) and non-homologous end joining (NHEJ). In non-homologous end joining repair, the two double-strand-breaks are directly ligated. In HR, the DNA ends are first resected in the 5' to 3' direction by nucleases; the resulting 3' single-stranded tails then invade the DNA double helix of a homologous, undamaged partner molecule, and are extended by the action of DNA polymerase, which copies information from the partner.

BRCA1, BRCA2, and Rad51

The function of *BRCA1* remains under active study. Many studies have proven that loss of BRCA1 protein results in defective DNA damage repair, abnormal centrosome duplication, cell-cycle arrest, growth retardation, increased apoptosis, genetic instability, and tumorigenesis, suggesting a role in DNA repair. Evidence is mounting that BRCA1 has local activities at double-stranded break (DSB) sites. Specifically, BRCA1 has a role in sensing DNA damage and checkpoint control of the cell cycle. It has been suggested that BRCA1's role in sensing breaks actually controls homology-directed DNA repair (23). In particular, BRCA1 is rapidly phosphorylated, by the kinases ATM and CHK2 after DNA damage in dividing cells, suggesting that it may work downstream of checkpoints that sense and signal DNA damage or problems with DNA replication during S phase (14-16). After phosphorylation, BRCA1 migrates to the site of the DSB, and the MRE11/RAD50/NBS1 complex is recruited (12). The exonucleolytic activity of the complex is mediated by MRE11 and is responsible for resecting DSB ends to generate ssDNA tracts (22). Recent work suggests that BRCA1 regulates the activity of this complex and under certain *in vitro* conditions, BRCA1 can inhibit the activity of MRE11 (19), regulating the length and the persistence of ssDNA generation at sites of DNA breakage.
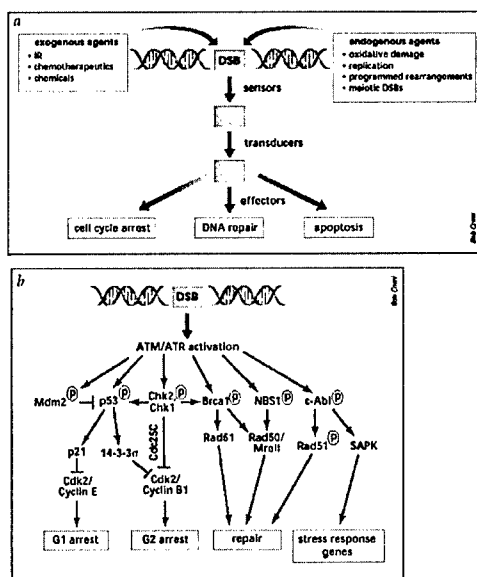
Figure 1 a) (17) The general organization of the DNA-damage response pathway. The presence of DSBs is recognized by a sensor, which transmits the signal to a series of downstream effector molecules through a transduction cascade, to activate signaling mechanisms for cell-cycle arrest and induction of repair, or cell death if the damage is irreparable. b) (17) ATM is activated in response to DSBs by an unknown mechanism. Activated ATM signals the presence of DNA damage by phosphorylating targets involved in cell-cycle arrest, DNA repair and stress response. In the DNA repair pathway, BRCA1 is phosphorylated and then signals BRCA2 (not shown), Rad51, Rad51/MRE11 to begin repair.

Additionally, interaction of BRCA1 with BRCA2 was suggested by the discovery that both proteins interact with RAD51 (20,21). Whereas it seems that RAD51 interacts with BRCA2 directly, the association of BRCA1 with RAD51 may be indirect, perhaps mediated by BRCA2 (21). The gene product of BRCA2 promotes and regulates the homologous recombination pathway of DNA double-strand-break repair (22-24). RAD51 forms foci in the nucleus after irradiation and catalyzes strand exchange, an early step in homologous recombination that results in the formation of heteroduplex DNA molecules (25). The three proteins (RAD51, BRCA1 and BRCA2) colocalize in irradiation induced foci (IRIF) and, notably, cells deficient in BRCA1 and BRCA2 are defective in RAD51 IRIF formation (26-29). Furthermore, it has also been shown that *BRCA1* deficient embryonic stem cells have decreased homologous recombinational repair (30).

BRCA2 has been linked to double-stranded DNA repair because of its interaction with Rad51, a RecA homologue in yeast whose function has been determined to be in homology-directed DNA repair. The regions that of BRCA2 that interact with Rad51 have been mapped to 8 BRC repeats (31-33). The 8 repeats in BRCA2 appear to be redundant for Rad51 binding, because any of the repeats bind Rad51 efficiently.

The relationship between BRCA1, BRCA2, and Rad51 is still actively being investigated, and in the last year, many studies have elucidated this complex's role in homologous recombinational repair. Because of the possible consequences of genetic polymorphisms in *BRCA1*, *BRCA2*, and *Rad51*, such as protein functional changes, this study will only focus on genetic polymorphisms of those genes.
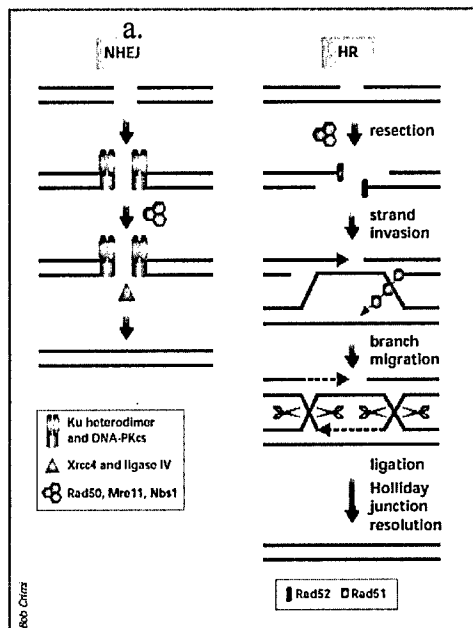

Figure 2a) (17): Pathways of DSB repair. NHEJ rejoins the two broken ends directly and generally leads to small DNA sequence deletions. It requires the DNA-end-binding protein Ku, which binds free DNA ends and recruits DNA-PKcs. Xrcc4 is then recruited along with DNA ligase IV. The Rad50-Mre11-Nbs1 complex, which contains helicase and exonuclease activities, may also function in NHEJ, particularly if the DNA ends require processing before ligation. HR requires Rad52, a DNA-end-binding protein, and Rad51, which forms filaments along the unwound DNA strand to facilitate strand invasion. The resected 3' end invades a homologous DNA duplex and is extended by DNA polymerase. In meiotic cells, the ends are ligated by DNA ligase I and the interwound DNA strands (Holliday junctions) are resolved resulting in either crossover or non-crossover gene conversion products. Only one of the many recombination products is shown here.

Figure 2b) (17): Components of DNA DSB repair pathways. NHEJ: Ku binds a DBS, followed by recruitment and activation of DNA-PKcs. XRCC4 and ligase IV are recruited directly or indirectly by the DNA-PK holoenzyme and/or are activated by DNA-PK-mediated phosphorylation. HR: proteins involved in mammals are indicated. The strand-exchange reaction catalyzed by Rad51 is facilitated by Rad52 through direct interaction. Rad54, a DNA-dependent ATPase, also interacts directly with Rad51 and stimulates its activity. Rad51-related proteins (Rad51B-D, Xrcc2 and Xrcc3) are also involved in HR. There is a direct interaction between Xrcc3 and Rad51, and Rad51B and Xrcc3 interact with Rad51C. Rad51 also interacts with Brca2 and indirectly with Brca1 through Brca2. The c-Abl tyrosine kinase modulates Rad51 strand exchange activity through phosphorylation. Brca1 and c-Abl are phosphorylated by ATM. The Mre11/Rad50/Nbs1 complex, which participates in both NHEJ and HR, is also indicated.

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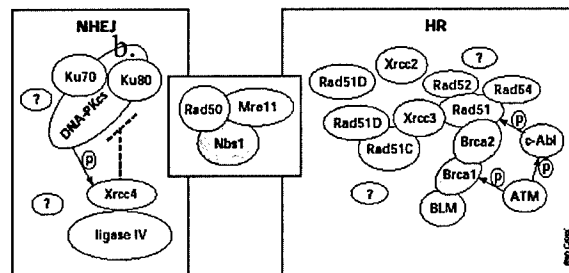
Mutagen Sensitivity Assay and DNA repair

Suboptimal or deficient repair of DNA damage may be a susceptibility factor predisposing women to breast cancer through increased sensitivity to carcinogenic damage from environmental exposures. The mutagen sensitivity assay, also known as the radiation-induced G2 chromatid assay, has been proposed as a phenotypic assay for cancer risk, including breast cancer (34). It is presumed that this assay is effective in measuring DNA repair capacity, at least in part. The assay measures defective DNA repair capacity by quantitating chromosomal breaks induced by gamma irradiation in cultured peripheral blood lymphocytes (PBL)(47,48). Although many mutagens such as bleomycin, x-rays, and UV light have been used in its place, gamma radiation has been used for breast cancer studies and triggers a variety of DNA damage relevant to this proposal.

This assay, in small studies has been applied to high-risk families. For example, in cancer-prone individuals, cells display an abnormally high, at least two-fold higher number of breaks (49). Also, Sanford et al have shown in several studies that more than 60 breaks and gaps per 100 metaphase cells, or 0.6 mean breaks and gaps per cell, is considered to be suboptimal repair (47,50). In another study, it was found that women at high risk (having 1 or more first-degree relative with breast cancer) were 5 times more likely than controls to have suboptimal DNA repair (OR=5.2, 1.04-28.57). Deficient DNA repair was found in all women with breast cancer, but in only 32% of control women (p=0.02)(51).

Several groups doing the mutagen sensitivity assay have found a significantly increased incidence of sensitivity compared with controls. In one of the largest epidemiological studies, it was found that when bleomycin (BLM) was used, 97% of hereditary breast cancer cases, 30% of their family members, 44% of sporadic cases, and 21% of controls were either sensitive or hypersensitive (>0.8 mean breaks per cell) (52). In other studies gamma radiation induced a higher degree of chromosomal damage in lymphocytes of family members of breast cancer patients compared with controls. In general, many other studies that have concluded ineffective DNA repair may be associated with cancer susceptibility in breast cancer patients and their first-degree relatives (53-58). Table 1 lists all of the mutagen sensitivity studies relevant to this study.

Table 1. Mutagen Sensitivity Studies

Author	Year	Mutagen	Normal (mbc)	Tumor (mbc)	Odds Ratios
Knight (56)	93	X-Ray	0.38-0.48	1.1-1.9	
Helzsouer	95	X-Ray	0.2-0.28	0.22-1.28	
Helzsouer (51)	96	X-Ray	0.16-0.5	1.1-1.6	
Parshad (55)	96	X-Ray	0.4	0.9-0.70	2.1-622.1
Patel (54)	97	X-Ray	0.6-0.92	1.05-1.59	6.9-23.8
Jyothish (52)	98	Bleomycin	0.58	0.8-1.28	1.3- 41.3
Roy (53)	2000	Bleomycin			4.1
Hsu (59)	85	Bleomycin	12%>1 MBC	60% >1 MBC	11.6
Scott (60)	94	X-Ray	0.94	1.09	6.9

Key Research Accomplishments, Reportable Outcomes, and Conclusions

Preliminary Experiments, Results, and Conclusions: Before starting, several preliminary experiments were performed. First, the conditions of the mutagen sensitivity assay were tested. To test if conditions of the mutagen sensitivity assay had to be changed, a hypotonic solution time response test (figure 3), colcemid dose and time response test (figure 4), and radiation dose response test was performed (figure 5 and 6).

Figure 3. The 25 minute treatment of 0.06M KCl produced no metaphases. a) The 35 and b)45 minute treatment of 0.06 KCl produced good, visible metaphases.
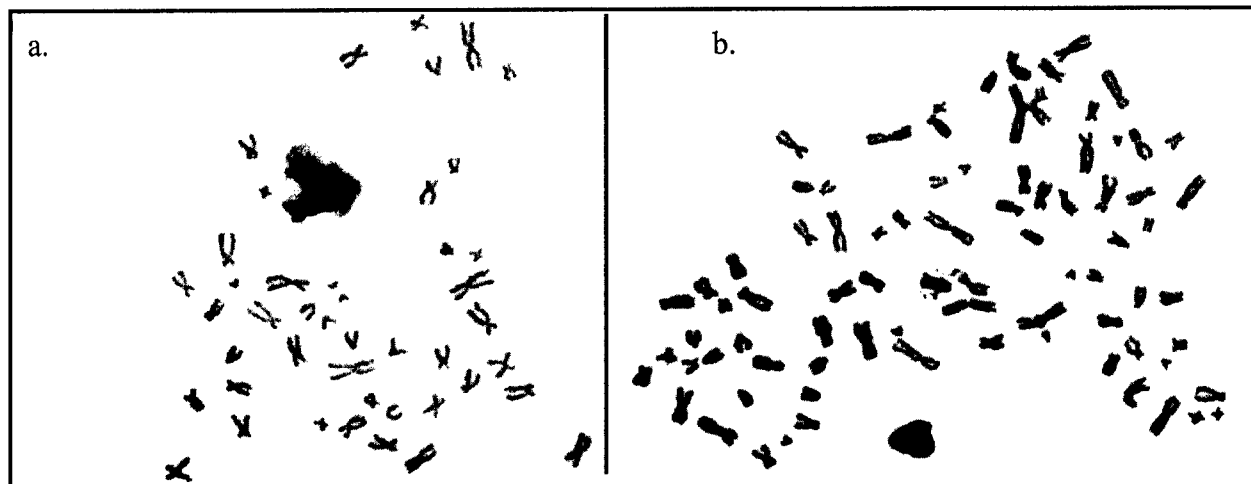
Figure 4. a) A dose of 10ug/uL at 2h and b) 20ug/uL caused chromosomes to be too condensed. c) A dose of 10ug/uL at 1.5 hours resulted in good metaphases.
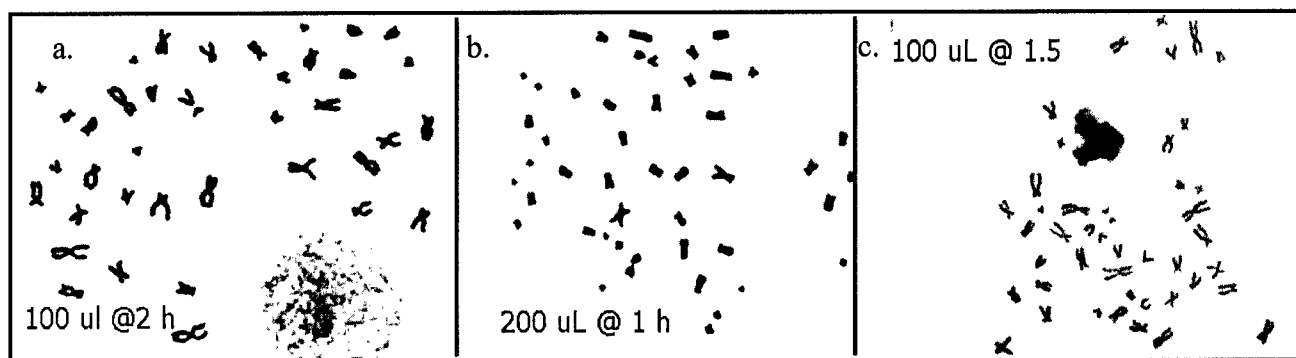
Figure 5. a) At 0Gy, there were many metaphases. b) At 1 Gy, there were few metaphases c and d) There were no metaphases found at 2 and 4 Gy.
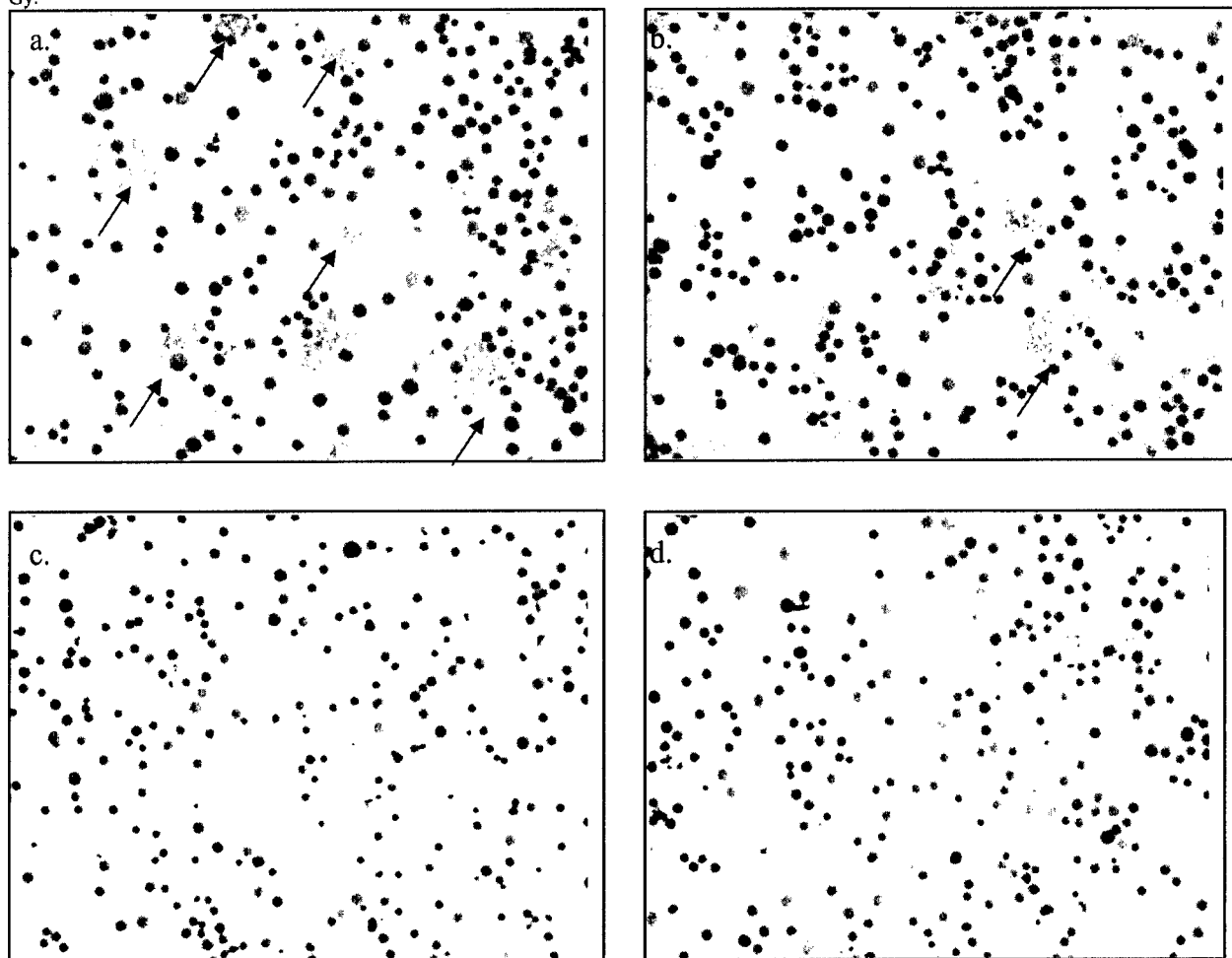
Figure 6. a) Eighty percent of the cells were still viable after an irradiation of 2Gy. b) The ideal amount of mean breaks per cell was found in the 1Gy irradiation. Irradiations at 2Gy produced to many breaks and at 4Gy, there were no viable cells. At 0.5 Gy, there were not enough breaks per cell.
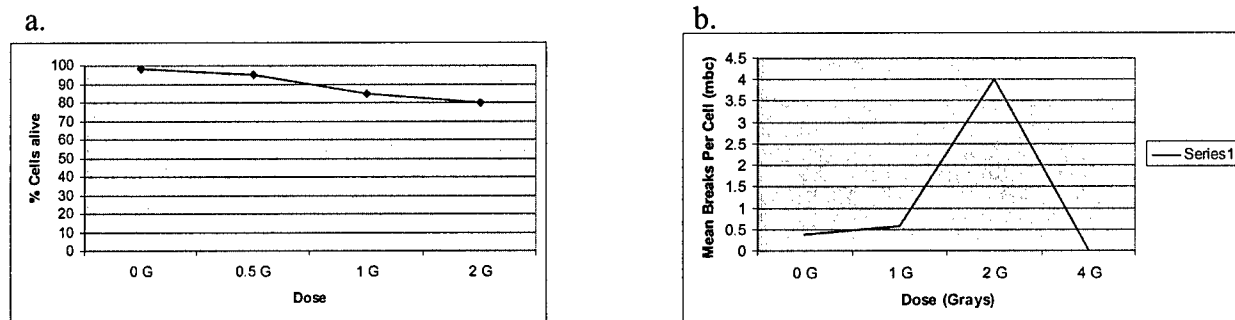
During the dose and time response experiments, cytogenetic training was also being provided by an expert cytogeneticist in the lab and slide readings in identical slides were

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compared between student and expert in a double-blinded experiment. Results from that experiment are described in the table 2.

Variations in slide readings within and between samples were also tested. Thirteen cell lines were tested in duplicate, triplicate, and quadruplicate. This was done in 2 ways: a) 2 sets of slides were made from the same culture; or cultures were grown on separate days, and mutagen sensitivity was performed on different days with different cultures. Either way, there was little intra-individual (within sample) variation, usually only different by 1 mean break per cell. There was some inter-individual (between samples), and adding to my hypothesis that there will be a range of mean breaks per cell between samples.

Table 2. Comparison of slide readings between expert cytogeneticist and trainee. Samples 1906-1 and 11597-1 will be repeated by both expert and student.

Slide Number	Student	Expert
9640-1	0.32	0.34
1793-1	0.22	0.24
7705-2	0.3	0.32
1906-1	0.22	
9790-1	No Good Metaphases	
11597-1	0.14	0.3
12005-1	0.24	0.22
12086-1	0.26	0.36
11596-1	0.24	0.22
12113-1	0.4	0.46
*All slides at 1G		

Figure 7. Mutagen Sensitivity Variability in EBV-immortalized Cell Lines from BRCA1 Mutation Carriers.
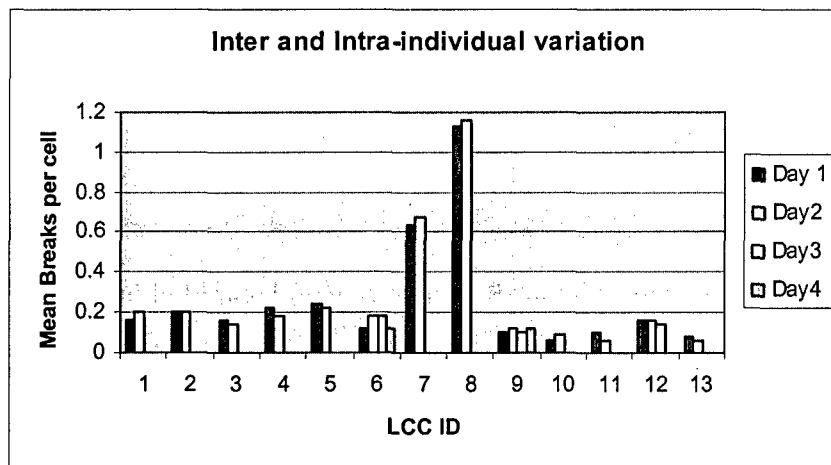
Observations were also made on 3 EBV cell lines obtained from tissue culture. BRCA1 negative unaffected (sample #1036) grew the fastest; BRCA1 healthy mutation carrier grew (sample #631) the 2nd fastest; and BRCA1 affected mutation carrier (sample #481) grew slowest. These observations agree with previous literature that loss of BRCA1 leads to apoptosis and inhibition of cell growth.

Conclusions for Preliminary Experiments
1. The mutagen sensitivity assay is reproducible
2. EBV-immortalized cell lines grow at different rates depending on mutation and cancer status
3. 35 min hypotonic solution treatment ideal
4. 10ug/ul Colcemid solution at 1.5 hours showed best metaphases and revealed best information
5. 1 Gray is best treatment for cells
6. Cytogenetic student can give reliable results

Aim 1: To identify DNA repair phenotypes in affected and healthy *BRCA1* mutation carriers that represent complex low penetrant genotypes by studying DNA repair proficiency in the terms of chromosomal breaks in EBV-immortalized lymphocytes following *in vitro* treatment with γ-irradiation.

Hypothesis 1: There will be a range of responses, i.e. inter-individual variation, for chromosomal breaks using gamma irradiation among affected and unaffected women with *BRCA1* mutations.

Subjects: The subjects in this study are confirmed BRCA1 mutation carriers recruited through the Lombardi Comprehensive Cancer Center –Familial Cancer Registry (LCCC-FCR). In this study, there are 99 affected and 54 are unaffected (healthy) *BRCA1* mutation carriers. Peripheral blood lymphocytes were immortalized by EBV and stored in liquid nitrogen by our tissue culture core. Thirty-six patients without founder mutations (187delAG and 5382insC) had complete *BRCA1* and *BRCA2* gene sequencing. Some patients are related (n=34). Patient demographics are listed on table 3. Figure 8 shows the age distribution in 65 unaffected and 95 healthy subjects.

Table 3. Patient Demographics.

Characteristic	With Cancer	Without Cancer	
	n=99	*n=54*	
Age Range (yr)	26-74	23-79	
Median Age (yr)	46	40	
Mean Age (yr)	46.12	44.35	p=0.43
Race			
White	91 (92%)	53 (98%)	
Black	5 (5%)	0	
Hispanic/Latino	2 (2%)	1 (2%)	
Unknown	1 (1%)	0	
Highest level of education completed			
High school graduate/ GED	7 (7%)	6 (11%)	
Some college/Technical school	13 (13%)	9 (17%)	
College graduate or beyond	78 (80%)	39 (72%)	
Cigarette Use			
Never	51 (53%)	35 (65%)	
Current	6 (7%)	5 (9%)	
Former	39 (40%)	14 (26%)	
Marital Status			
Single or Never married	11 (11%)	3 (6%)	
Married or Living together	76 (77%)	45 (83%)	
Divorced	8 (8%)	3 (6%)	
Separated	2 (2%)	0	
Widowed	2 (2%)	3 (6%)	
Mutation			
185delAG (BRCA1)	32 (32%)	22 (41%)	
5382insC (BRCA1)	14 (14%)	7 (13%)	
Other	53 (54%)	25 (46%)	

Figure 8. Age frequencies in affected and healthy BRCA1 mutation carriers.

P=0.43	N	Mean	Std. Dev.
Unaffected	54	44.35	14.02
Affected	99	46.12	1.2
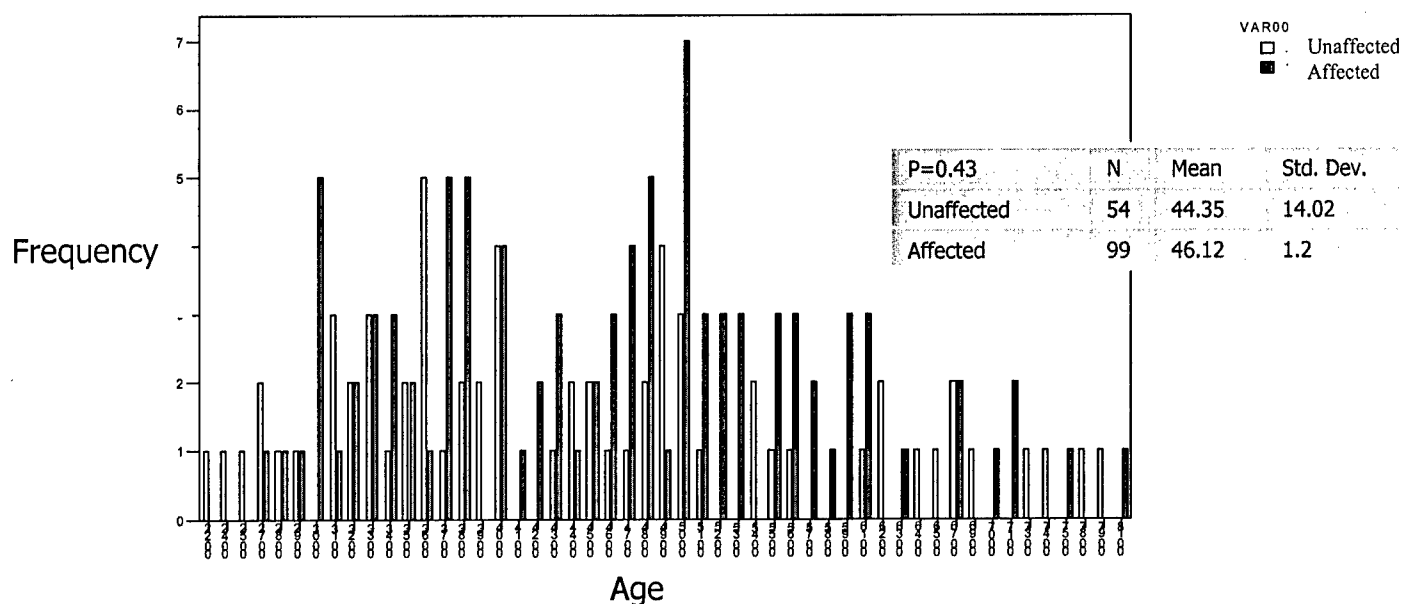
Cell Lines: To date, 152 ebv-immortalized cell lines from *BRCA1* mutation carriers have been received, labeled, and logged in, in a secure database. Of those 152, 10 have died, but will be received at a later time. Along with the 152 cell lines, 19 fresh blood samples have also been received, labeled, and logged in, in a secure database. Of the 19 fresh blood samples, 9 corresponding cell lines have been collected for the comparison between fresh blood and ebv-immortalized cell line mutagen sensitivity. The other 10 corresponding ebv-immortalized cell-lines are undergoing transformation. See appendix A for list of cell lines received.
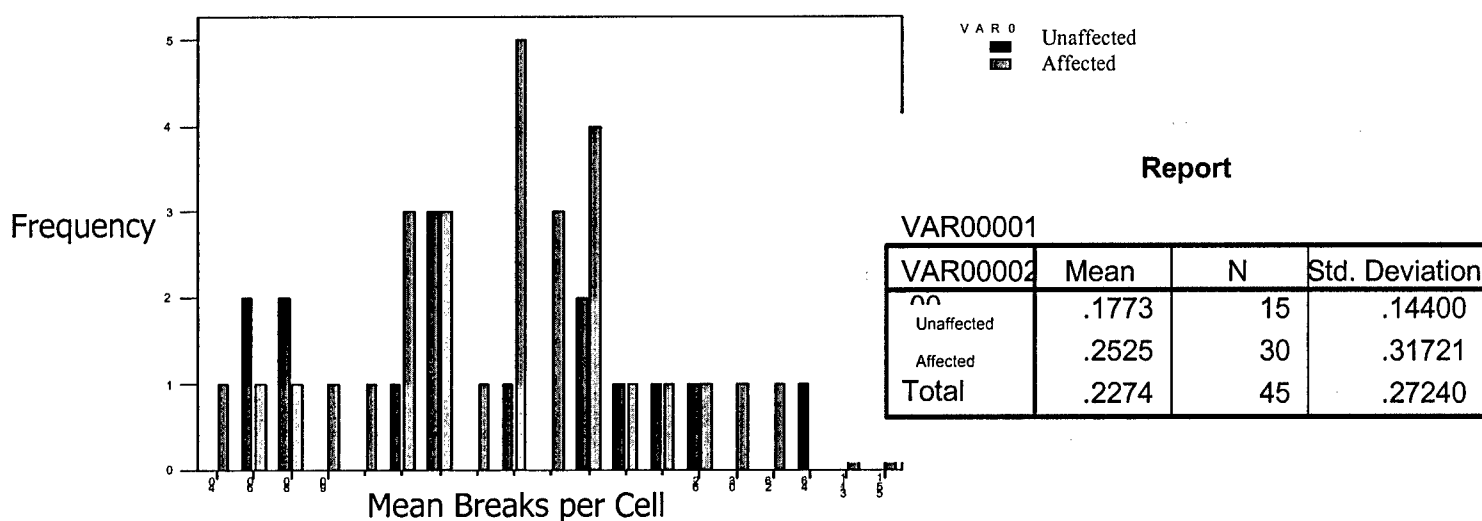
Mutagen Sensitivity: This project began by characterizing DNA repair capacity in healthy and affected subjects using immortalized lymphocytes and determining their sensitivity to mutagen exposure. The assay is called "mutagen sensitivity assay" and it is a marker of DNA repair. The mutagen sensitivity assay has been performed on 142 ebv-immortalized cell lines and all 19 fresh blood samples. In short, 1Gy radiation from a ^{137}Cs source gamma irradiator was used to induce chromosomal breakage according to the method developed by Sanford and Parshad (47,62). The culture medium used was RPMI1640 with L-glutamine. Fresh media was made once a week. Cultures were incubated for 4 hours to allow time for repair of breaks induced in the chromosomes. The cultures were then treated with colcemid to accumulate mitosis before harvesting. The cultures were then fixed, 4 slides per sample prepared (2-OGy and 2-1Gy), and stained with Giemsa. Chromosomal breaks were scored by microscopy. The frequency of chromatid breaks per cell (b/c) was calculated as a measure of an individual's DNA repair efficiency. Only chromatid breaks (discontinuity longer that the chromatid width) was scored. A minimum of 25 and a maximum of 50 metaphases per culture were scored. A diagrammatic representation of a few types gamma radiation-induced chromosomal breaks are shown in Figure 9. The polymorphisms that are found to affect mutagen sensitivity will be used to determine if there is indeed a genotype that is associated with breast cancer risk.

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Figure 9. Diagrammatic representation of a few types of gamma radiation-induced chromosomal damage.
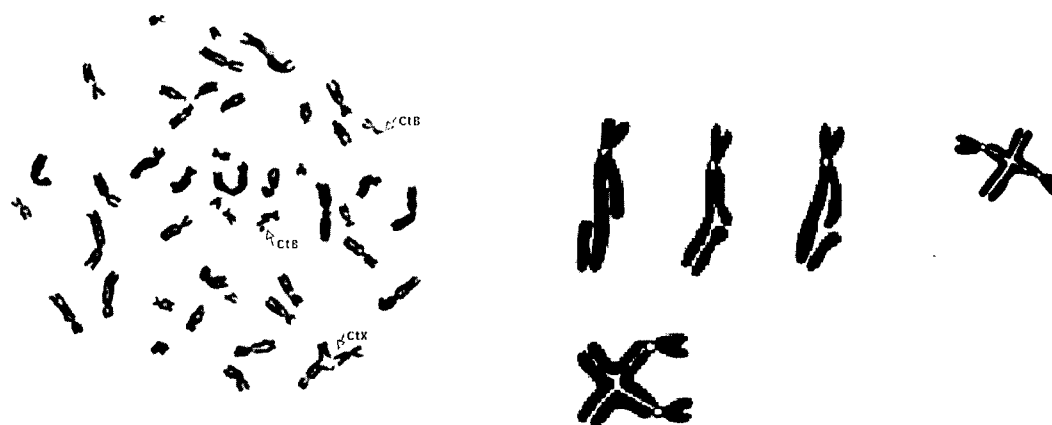
Results and Statistical Analysis: The distribution of chromatid breaks are described in figure 10. A frequency graph of 45 samples was plotted against the number of breaks. Only data analyzed is included in the figure. The independent variable is the frequency of mean breaks per cell and the dependent variable is breast cancer being affected or healthy and frequency.

Figure 10. a) Frequency of mean breaks per cell in 45 cell lines in affected BRCA1 mutation carriers compared to healthy BRCA1 mutation carriers. b) Mean, sample size, and standard deviation of frequency of mean breaks per cell in unaffected vs. affected subjects.

Report

VAR00001

VAR00002	Mean	N	Std. Deviation
Unaffected	.1773	15	.14400
Affected	.2525	30	.31721
Total	.2274	45	.27240

Conclusions for Hypothesis 1: There are a range of responses in the patient population which agrees with hypothesis 1 so far. A trend indicates affected BRCA1 mutation carriers have higher mean breaks per cell than healthy mutation carriers, but need larger sample size to be significant (n=69 in each group to detect difference).

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Aim 2: To identify single nucleotide polymorphisms (SNPs) in *BRCA1, BRCA2,* and *Rad5*1 that are associated with defective DNA repair capacity in EBV-immortalized lymphocytes.

Hypothesis 2: There will be polymorphisms in DNA repair genes (*BRCA1, BRCA2, Rad51*) that will affect the number of chromosomal breaks in affected and healthy *BRCA1* mutation carriers

Genotype analysis: Polymorphisms worth characterizing and analyzing were identified in a preliminary study of sequencing data by Myriad Genetic Laboratories, Inc. in 36 patients without founder mutations (187delAG and 5382insC) and from the scientific literature and various web-based resources such as NIH's dbSNP and SNP500. PCR-based genotyping assays will be developed for *BRCA1*'s P871L, E1038G, S1613G, K1183R, and BRCA2's H372N polymorphisms (see Table 4). SNP discovery in Rad51 will be done by direct sequencing of all 10 exons. These SNPs were chosen to be analyzed because they were the most frequently found SNPs in 36 *BRCA1* mutation carriers and because they were found in an important region were an amino acid change could lead to change in the structure and function of the protein. For example, a couple *BRCA1*'s and *BRCA2*'s SNPs were found in the BRCT repeats of *BRCA1* and *BRC* repeats of BRCA2, where Rad51 binds.

Table 4. Frequency of polymorphisms found in 36 subjects, amino acid changes, and characteristic changes.

N=36	polymorphism	Frequency	AA change	Characteristic change	Assay
BRCA1	P871L	47%	Pro→Leu	N, hydrophobic non-polar	
	E1038G	42%	GluAcid→Gly	Y, hydrophilic polar→un-charged polar	RFLP,NlaIV will cut if SNP present
	S1613G	39%	Ser→Gly	N, Uncharged polar	
	K1183R	36%	Lys→Arg	N, hydrophilic polar	
BRCA2	H372N	86%	His→ Asn	Y, hydrophilic polar→un-charged polar	sequencing
Rad51	5'UTR, 135	n/a	n/a	n/a	RFLP, MVAI will cut if SNP present
	Many	n/a	YES	YES	Sequencing all exons

DNA Extractions: DNA from 142 cell culture pellets has been extracted by the Qiagen M48 Biorobot (figure 11). In short, cell culture pellets were collected, washed in PBS, and stored in -20°C until time for extraction. All of the samples were carefully labeled and logged into a secure database. One day before extraction, approximately $2.0x10^6$ cells were digested with 10uL of proteinase K, to degrade unwanted proteins and incubated in a 55°C water bath. After digestion, 150uL of PBS was added and samples were loaded in the M48 Biorobot (48 samples at a time). After extraction, DNA samples were quantitated by Genequant or Spectramax. Acceptable DNA:Protein (ratios) are 1.7-1.9, and if the quality was found to be outside these parameters, DNA was extracted again. After DNA was quantitated, aliquots of 50ug/ul, 10ug/ul, and 2ug/ul were made, labeled and logged into a secure database.
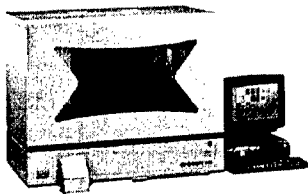
Figure 11. Qiagen M48 Biorobot.

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Challenges and Obstacles: Some of the challenges faced were found when it came to the quality of DNA. Initially, my cell pellets were too large and contained too many cells (5.0×10^6 - 10.0×10^6 cells). After troubleshooting with Qiagen, I found out that there was a supplemental protocol, not yet obtained by our lab, that described that the amount of cells the M48 biorobot would be most effective at extracting. Extract DNA from less than 2.0×10^6 cells, resulted in the at the highest quality of DNA.

Statistical Analysis of Genotyping Data: With the sequencing data obtained from Myriad, preliminary analyses of genotype-phenotype correlations were done. The association between genotype and the mean number of breaks per cell have been calculated by unconditional logistic regression and odds ratio of relative risk with 95% confidence intervals was calculated. Results of logistic regression analyses can be seen in table 5. Later, they will be adjusted for confounders. If these results cannot be normalized, the chi-squared test will be used used. SNPs to further assess in a case-control study will be determined by the results of statistical analysis.

Table 5. Relationship between genetic polymorphisms and mutations in *BRCA1* and *BRCA2* and mutagen sensitivity among BRCA1 mutation carriers.

| Gene	Mutagen Sensitivity (MBC)			
	Hi* (N=27)	Lo (N=18)	OR	95% CI
5382insC				
Wildtype	23	17	1.0	
Variant	4	1	**3.0**	**0.3 - 29.8**
185delAG				
Wildtype	21	14	1.0	
Variant	6	4	1.0	0.0 - 6.0
	Hi* (N=15)	Lo (N=3)	OR	95% CI
H372N				
Wildtype	1	1	1.0	
Variant	14	2	**7.0**	**0.3 - 140.0**
K1183R				
Wildtype	8	2	1.0	
Variant	7	1	**1.8**	**0.1 - 17.7**
P871L				
Wildtype	7	1	1.0	
Variant	8	2	0.6	0.0 - 6.0
E1038G				
Wildtype	8	1	1.0	
Variant	7	5	0.4	0 - 4.4
S1613G				
Wildtype	8	1	1.0	
Variant	7	5	0.4	0 - 4.4
*Hi>0.12				

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Sequencing of Rad51: PCR and sequencing is currently being performed on all 10 Rad51 exons. VariantSEQr Resequencing System primers were purchased from Applied Biosystems. The system is designed for resequencing the exons of genes implicated in cancer and various other complex diseases. VariantSEQr Resequencing System provides ready to use primer sets and a universal protocol for PCR, sequencing, and data analysis. In short, genomic DNA was amplified with a primer set, Amplitaq Gold PCR Master mix, ddH20, and 50% glycerol. The thermal cycler was programmed for activation at 96°C for 5 minutes, 40 cycles of amplification at 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds. The final extension was done at 72°C for 10 minutes. After amplification, the sample is held at 4°C indefinitely.

After the PCR, the reaction was cleaned up using ExoSAP-it. In short, 4ul of ExoSAP-it was added to 10uL of PCR reaction. Then the reaction was centrifuged and placed in the thermal cycler for a program of 37°C for 30 minutes, 80°C for 15 minutes, and sample is held at 4°C indefinitely. After clean-up, the DNA is quantitated by running 3ul of sample against a DNA mass ladder on 2% agarose.

For sequencing, BigDye Terminator Reaction mix is added to 100ng/ul of clean DNA, *M13* universal forward primer, and dH20. For this PCR reaction, the thermal cycler conditions are as follow: Denaturation at 96°C for 1 minute, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. After amplification, the sample was held at 4°C indefinitely.

Reactions were then purified by spinning in a 96-well sephadex spin column. After purification, samples were then loaded into the MagaBace capillary sequencer (Figure 12), where specific parameters are loaded for each reaction plate. Results were analyzed initially using Bioedit, free software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), but will later be analysed using Sequencher.

Figure 12. Amersham MegaBace Capillary sequencer.

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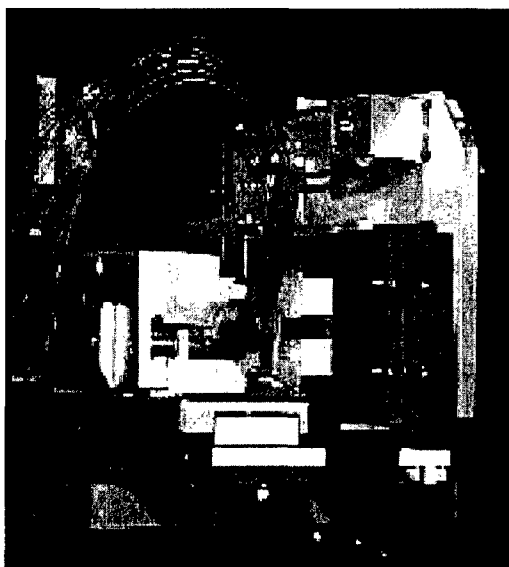
Results of Sequencing: I successfully amplified 5 exons of Rad51 in 96 samples (Figure 13). After many tries, the ABI protocol was optimized for the MegaBace capillary sequencer. Because my fragments are small (<300bp), a DNA concentration titration resulted in less DNA being used (25-50ng). At 25ng/uL, good results were obtained on the MegaBace capillary sequencer (Figure 14).

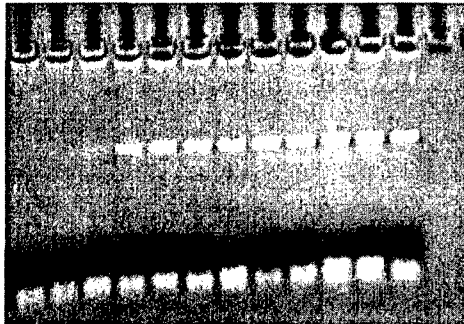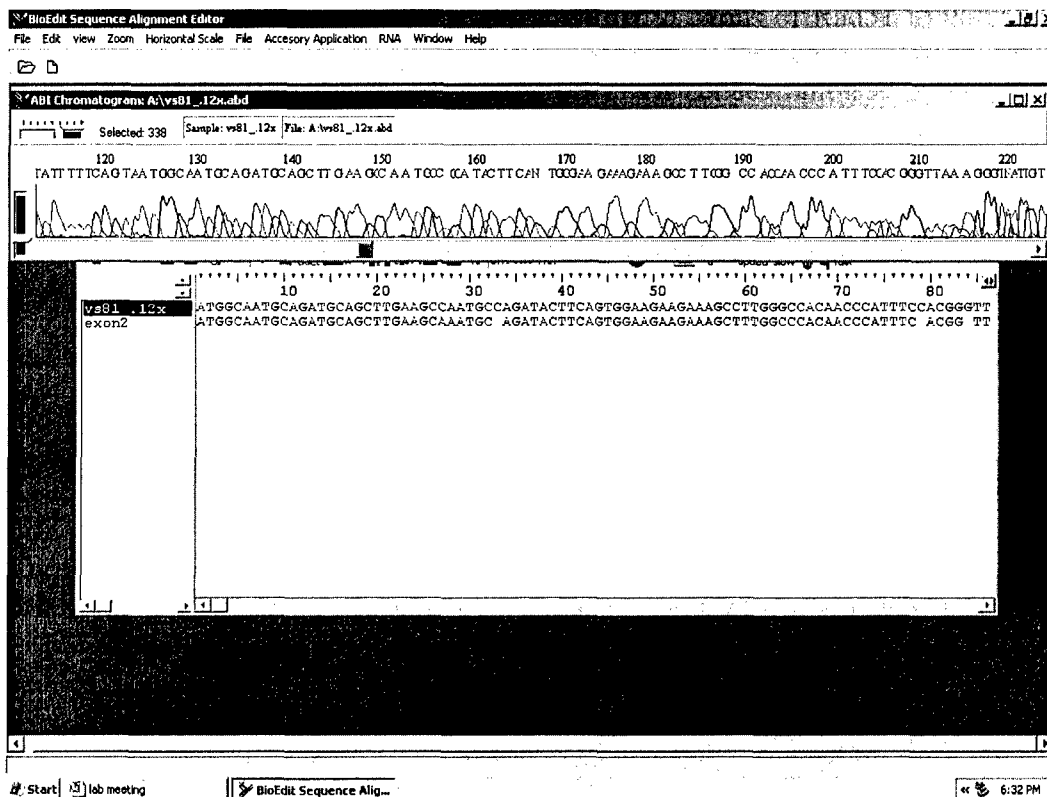**Figure 13.** Exon 2 amplification.

Figure 14. Comparison of sequencing of Exon 2 in sample vs81 to Genebank's Rad51's exon 2.

Aim 3: To identify single nucleotide polymorphisms (SNPs) in *BRCA1, BRCA2,* and *Rad51* associated with increased risk to sporadic breast cancer. (This aim cannot be worked until aim 2 is complete).

Hypothesis 3: Some of the single nucleotide polymorphisms shown in the previous aim to increase risk in hereditary breast cancer will be the same polymorphisms found to increase risk in cases versus controls.

Subjects: Dr. Jo Freudenheim at the State University of New York at Buffalo, our collaborator, has enrolled 1165 breast cancer cases and 2170 controls in a breast cancer and alcohol case-control study. The women are all residents of Erie and Niagara counties, New York. Cases are women with incident breast cancer between the ages of 35-79 years. Controls were randomly selected from residents of Erie and Niagara Counties using lists provided by the New York State Department of Motor Vehicles driver's License enrollees for those less than 65 years of age, and the Health care Finance Administration for those 65 years of age and older. Controls were frequency matched by age, sex, race, and county of residences to cases. Blood and oral rinses from cases and controls have been obtained and DNA has been extracted by phenol-chloroform methods. DNA aliquots were made labeled, logged in a secure database, and store in -20°C.

Genotyping: Polymorphisms identified in hereditary cases (aim 2) will be used to genotype in this epidemiological case-control study. Assays will be developed and genotyping will be done by RFLP or direct sequencing. Twenty percent of the samples will be repeated for quality control.

Statistical Analysis: Allele frequencies in cases vs. control subjects will be compared using the chi squared test and a test of Hardy Weinberg equilibrium. The association of disease status and polymorphisms will be analyzed using unconditional logistic regression. Odds ratio estimates of relative risk with 95% confidence intervals will be used in the statistical analysis of the data and will be adjusted for confounders.

Secondary Aim:
Aim 1: To compare the number of induced chromosomal breaks in EBV-immortalized and freshly cultured lymphocytes from affected and unaffected *BRCA1* carriers.

Hypothesis 1: Mutagen sensitivity in freshly cultured lymphocytes will be correlated with mutagen sensitivity in immortalized lymphocytes from the same women.

Results and Conclusions: I have not received enough corresponding ebv-immortalized cell lines to make this comparison or analysis. I have only received 9 cell lines to 19 fresh bloods.

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Appendix A: All samples received from tissue culture core (n=152).

946	813	294	116	114	115	629	1166
947	846/843	626	867	573	654	536	1169
1016	847	28	868	226	863	31	10
1029	824	128	963	577	344	18	25
184	651	149	941	21	373	39	140
187	657	252	1019	24	424	58	158
191	669	397	40	37	430	66	164
214	772	406	201	46	431	94	996
901	526	155	249	45	435	130	243
924	607	16	755	52	446	193	243/RGP1210
840	618	135	853	60	459	208	127
937	656	9890	966	154	481	222	30
794	99	524	968	182	488	223	
787	177	139	985	183	507	247	
653	463	828	999	211	528	452	
589	612	825	1009	236	551	533	
727	639	285	1011	242	574	1079	
830	665	79	251	315	578	1159	
721	668	125	416	325	592	1160	
693	737	126	259	333	598/698	1161	

DNA repair gene polymorphisms in hereditary and sporadic breast cancer

Introduction: Breast cancer risk for both sporadic and familial breast cancer can better be elucidated in studies in women with *BRCA1* mutations or in women in high risk breast/ovarian cancer families. Since the BRCA1 protein is involved in DNA repair, genotype-phenotype relationships can be established by the assessment of DNA repair efficiency and the identification of genetic polymorphisms in the BRCA1 DNA repair pathway. **Objective:** To study risks associated with certain polymorphisms in DNA repair genes, EBV-immortalized cells from women in the Lombardi Cancer Center Familial Registry were used in both a phenotypic assay that measures DNA repair capacity and genotypic assays that determined genotypes that increased susceptibility to breast cancer. **Study Population:** Ninety-nine affected and 54 unaffected *BRCA1* mutation carriers were analyzed in this study. There mean ages are 44 and 46, respectively (p=0.43). **Methods:** Gamma radiation was used to induce chromosomal breaks and those chromosomal breaks were scored, counted and compared within each patient and between patients. Also, the *BRCA1, BRCA2,* and *Rad51* genes were sequenced and the genotypes whose polymorphisms code for amino acid changes in conserved and functional areas were analyzed. We predict that polymorphisms in these genes will be highly correlated with deficient DNA repair in high risk breast cancer families. **Results:** We have found a difference in mean breaks per cell in cases versus controls. Also, several *BRCA1* and *BRCA2* polymorphisms appear to be associated with decreased DNA repair efficiency or high mean breaks per cell in our population. Specifically, the N372H (OR: 7.0 CI: 0.3-140.0) polymorphism in BRCA2, the K1183R (OR:1.8 CI:0.1-17.7) polymorphism in BRCA1, and the 5382insC mutation/ polymorphism (OR:3.0 CI:0.3-29.8), appear to be correlated with mutagen sensitivity, but a larger sample size is needed for odds ratios to be significant. In addition, we have a large population of 187delAG (n=54) and 5382insC (n=21) mutation carriers, so we can begin to look at what polymorphisms will affect risk within those mutation carriers. **Conclusions:** Ultimately, the study of the high risk families can inform, and set priorities for the study of genetic polymorphisms in sporadic breast cancer studies. These studies will identify susceptible groups of women and aid in the development of more rationale prevention strategies.

Classes taken:

July 2004-Aug. 2004 **NCI Summer Curriculum in Cancer Prevention**   Rockville, MD
- The first part of the course, Principles and Practice of Cancer prevention and control, focused on concepts, methods, issues, and applications related to the field. The learning modules in the first part included: Ethics, law and policy; Diet; Biometric methodology; Prevention and Control of Organ Specific Tumors; Behavioral Science and Community Intervention; Health Disparities and Cancer Prevention in Diverse Populations; And Occupational Cancer.
- The second part, Molecular Prevention, focused on concepts, methods, issues, and applications of molecular biology in cancer prevention efforts and the genetics of cancer. Learned about basic laboratory methodology and theory of how molecular techniques are applied to molecular epidemiology, bionutrition, chemoprevention, biomarkers, and translational research.

May 2003- July 2004 **Johns Hopkins School of Public Health**   Baltimore, MD
- Graduate of summer program in Epidemiology and Biostatistics Graduate of Summer program in Epidemiology and Biostatistics.
- Classes taken include Introduction to Epidemiology, Biostatistics and Genetic Epidemiology in Populations

May 29-30, 2003   **Georgetown University School of Nursing**   Washington, DC
                       & Health Studies
- This conference focused on the epidemiology of tobacco use, nicotine pharmacology, principles of addiction, drug interactions with smoking, and assisting patients with quitting.

Conferences attended:

American Public Health Association Annual Conference	November 2004
American Association for Cancer Research Annual Conference	April 2004
American Association for Cancer Research- Radiation Biology and Cancer	February 2004
American Association for Cancer Research- SNPs, Haplotypes, and Cancer	November 2003
Lombardi Cancer Center Research Fair –presented	2002-2005

"DNA repair gene polymorphisms in Hereditary and Sporadic Breast Cancer